Researchers develop alternative to GFP markers

A new cell-marking technique that could prove cheaper and easier to use than existing methods, has been developed by US researchers.

The technique exploits the binding properties of chitinase, enzymes that breakdown chitin in funghi and various animal exo-skeletons, and could be used in myriad cell-sorting applications.

The best-known cell marker in use today is green fluorescent protein - GFP - a jellyfish protein that emits green light when illuminated by blue to ultraviolet light.

But while widely used in many fluorescence-based cell sorting applications, the technique can be expensive and cumbersome. Alternative cell-marking techniques use marker molecules to which antibodies or metals will bind tightly, but these can cause unwanted side effects on the cells that they mark.

Professor Richard Lerner and Dr Yingjie Peng from The Scripps Research Institute, and colleagues, have developed a new technique to mark cells, based on the expression of the chitinase enzyme's chitin-binding domain (ChBD) on a cell surface.

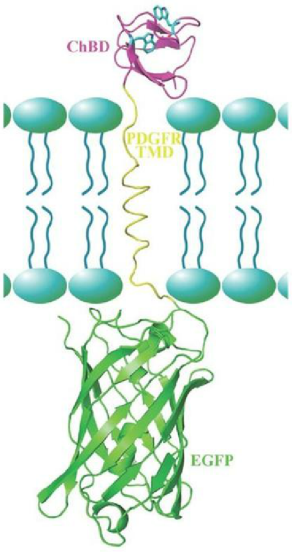
The technique exploits the "irreversible" binding between ChBD and its ligand chitin. While this bond has already been used as a marker system for selecting ChBD-tagged proteins in a lab dish, the researchers actually use the marker system to mark and select cells. In the basic technique, a new gene is added to cells within a larger DNA vector that also includes the genetic sequences for ChBD and GFP. Once produced, the ChBD molecule is held to the outer surface of the host cell's plasma membrane while the GFP molecule sits just inside the membrane.

In this way, the GFP within the cell serves as a visual beacon, while the ChBD on the cell surface and linked to the GFP via a protein spanning the plasma membrane, acts as a "gripping point" for cell selection.

"The expression of ChBD on an eukaryotic cell surface gives those cells orthogonal selectability. This helps to capture target cells from a larger population and select just the membrane fraction of a sample of cellular material," said Peng.

Peng and his colleagues have demonstrated this using chitin-coated magnetic beads, and a magnet to quickly isolate a fraction of membrane material from ChBD-marked test cells.

Research is published in *Angewandte Chemie International.*



The ChBD is linked to enhanced green fluorescent protein, through a protein, that spans the plasma membrane
The Scripps Research Institute

Reconstruction algorithm boosts EM imaging

By developing a novel MBIR algorithm for bright-field electron tomography, Professor Charles Bouman from the University of Purdue and colleagues, have reconstructed 3D models of aluminium nano-particles from noisy data.

"Before now, researchers haven't performed bright-field reconstruction on crystalline samples such as these, as Bragg diffraction leads to anomalous data measurements, making the reconstructions terrible,' said Bouman.

Reconstructions of crystalline samples using standard algorithms, such as Filtered Back Projection, typically contain blurring and streaks. However, the new algorithm rejects measurements that deviate significantly from Beer's Law - relating the absorption of light through a material, to the material's properties - giving a clearer image.

And as Bouman adds, the method incorporates models of the microscope as well as statistics of the sample and any noise.

"We can account for many non-ideal characteristics of an electron microscope such as limited view angle, attenuation, diffraction, bias and gain," he says.

The researchers have previously applied their method to medical CT reconstruction. GE Healthcare now includes the reconstruction technique in its CT scanners to boost resolutions and reduce artifacts that could hide underlying structures. Research was presented at Electronic Imaging 2013, SPIE.

Fluorescence microscopy images mitosis in fly embryo extract

Claiming a world first, researchers at Germany-based EMBL have used confocal fluorescence microscopy to capture successive rounds of cell division within single fruit fly embryo extracts.

This process typically takes place deep inside a fruit fly embryo, making live microscopy a challenge, but Dr Ivo Telley and colleagues believe their ex-vivo method will help to unravel the complex mechanisms behind cell division.

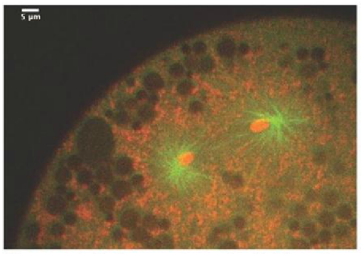
To obtain a sample of dividing nuclei, the researchers first immobilised the fruit fly embryos on a cover glass, which was then placed on an inverted microscope, equipped with motorised manipulators.

Cytoplasm was extracted from the embryo with pipettes - held by the manipulators - and ejected onto the glass, ready for analysis.

"All manipulation was performed using polarised bright-field illumination... and high efficient instrument control was needed for success," explains Telley. The cytoplasm extract contained dozens of autonomously dividing nuclei, which the researchers could image, ex vivo, within sixty and ninety minutes of embryo collection.

"We monitored [mitosis] using time-lapse fluorescence microscopy with DNA markers or microtubules. The trick is first to choose the right timing; embryos cycle through nuclear divisions every eight to nine minutes," says Telley. The researchers assert that studying nuclear division in their membrane-free system opens the door to more micro-biomechanical studies of a cell's 'mitotic machinery'.

They now hope to use correlative fluorescence and electron microscopy imaging to study the fruit fly extracts as well as carry out fluorescence imaging of cell cycle regulation of the first rapid divisions.



The new technique captures the division of membrane-less cells. Red: chromosomes, Green: spindles; a cell's network of filaments
Credit: Ivo Telley/EMBL